## THE PIGMENT OF THE VERTEBRATE LENS

A FEW years ago the late H. D. Judd and the writer described the yellow lenses of squirrels, snakes and lampreys.[1] The yellow coloration constitutes one of several types of intra-ocular filters which are widespread among diurnal vertebrates and have a quadruple effect in promoting visual acuity.

At that time it was suggested that yellow lenses might be found in certain other animals, among them the tree-shrews (*Tupaia*), the strongly diurnal geckoes, *Phelsuma* and *Lygodactylus,* and the hyrax, *Procavia.* Some of these predictions have since been fulfilled by investigators whose cooperation is deeply appreciated:

Dr. Hugh M. Smith, of Bangkok, Thai, compared the lens of an adult *Tupaia belangeri* with the "Noviol O" glass which matches the lens of the average sciurid species, and judged it to be "about halfway between Noviol O and colorless."

Mr. Arthur Loveridge, of the Harvard Museum of Comparative Zoology, examined the lens of an adult *Lygodactylus picturatus* on Manda Island, Uganda, and reported to the writer that it was pale yellow.

*Procavia* seems to be nocturnal rather than diurnal as we had been led to believe; but, on the other hand, the American beaver appears to be fundamentally diurnal or indifferent to night and day, not strictly nocturnal as usually described, and might be expected to have a yellow lens. Accounts of old travelers and recent statements by those familiar with the animal in wild regions indicate that the beaver has readily become nocturnal wherever it is in even light contact with civilization, but is diurnal when quite undisturbed. Protected beavers in such sanctuaries as the national parks have slowly reverted to diurnality in recent years.

A large (50 lb.) *Castor canadensis* obtained by courtesy of the Michigan Department of Conservation proved, however, to have colorless lenses. The beaver retina has not yet been studied histologically, but the small amount of rhodopsin present after thorough dark-adaptation indicates that it contains rods, though these are probably small or small in numbers. The species thus has a twenty-four-hour eye and, having avoided such restrictive specializations as a yellow lens, is able to become nocturnal when it must.

The vertebrate lens pigment—possibly a closely knit group of compounds rather than a single one—has been named "lentiflavin"[1] and was found to be readily extractible only with alkali. Since most melanins (though apparently not ocular melanins) are alkali-insoluble this ambiguous behavior stalemated our attempts at chemical identification and led us to hope for a further clue from the study of albinos.

Some time ago Dr. S. A. Houchen, of Olney, Illinois, kindly examined for us the lenses of a two-year-old member of the famous Olney population of albino gray squirrels (*Sciurus carolinensis leucotis*). He pronounced them "a trifle lighter" than Noviol O. This describes the normal gray squirrel lens; but since the writer could not supply Dr. Houchen with a glass sample exactly matching the latter, it was not certain whether the albino might not show some reduction in pigmentation.

This uncertainty was recently removed when the writer obtained an albino woodchuck, *Marmota monax.* The specimen had one patch of light color on the head and some in the tail, but otherwise (and as regards the eyes) was a perfect albino. The lens proved to be exactly matched by Noviol O, as is that of the normal woodchuck.

The yellow coloration of vertebrate lenses is thus certainly not due to sparse melanin, as in normal human adult and early cataractous lenses. It does not seem to be known whether "melanoid" pigments (which are alkali-soluble) can be formed by albinos. Lentiflavin does not appear to be a carotenoid, an anthocyanin or a flavone, and perhaps represents a hitherto unknown group of animal pigments. Further attack upon the problem of its chemical nature can best be made by investigators living where ground-squirrels (*Citellus* spp.) abound, since in their lenses the pigmentation is rich enough to yield an adequate KOH extract with a minimum of collecting effort.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## AN APPARATUS FOR THE STUDY OF EXPERIMENTAL AIR-BORNE DISEASE[1]

AN apparatus for the study of experimental air-borne disease, developed in the Laboratories for the Study of Air-borne Infection, consists essentially of three parts: (1) a tight chamber in which to subject animals to a controlled infected atmosphere, (2) a special atomizer which delivers a fine stream of droplet

[1] G. L. Walls and H. D. Judd, *Brit. Jour. Ophthalmol.,* 17: 641–75 and 705–25, 1933.

[1] This study is supported by a grant from the Commonwealth Fund to the University of Pennsylvania for investigations on air-borne infection, with laboratories in the Department of Bacteriology, the Children's Hospital of Philadelphia and the Henry Phipps Institute for the Study, Treatment and Prevention of Tuberculosis.

nuclei derived from cultures of test organisms, and (3) an incinerator providing the influent draft as well as incinerating the effluent air from the animal chamber.

The apparatus, except at the entrance of the influent duct, where the atomized culture is drawn into the influent air stream, works under negative pressure. Flow of air and nuclei through the chamber is uniform. Dosage is regulated by varying the concentration of the fluid from which the nuclei are derived and by varying the time of exposure of the animals within the chamber. Treatment of the air entering the chamber, such as passage through long ducts or treatment with ultra-violet light, is permitted.

Quantitative features of utmost importance in studies of experimental pathology have been proven for the apparatus under severe experience. Dosage is uniform and determinate. Samples taken with the air centrifuge exhausted into the incinerating chimney have independently determined the constancy of the predetermined air infection.

Penetration of droplet nuclei to all the lobes establishes this quantitative method of lung inoculation. Studies showing the importance of dosage in determining the characteristics of pathologic or epidemiologic patterns with virus as well as with acute and chronic bacterial infections will be reported later with collaborators in the special fields.

Quantitative relationship between dosage and mouse mortality observed with air-borne B streptococci is conspicuously absent with air-borne pneumococcus. Compound infection of mice with pneumococcus and streptococcus exceeds the sum of infections with either. Promising experiments on influenza virus are now being conducted. Development of discrete tubercles in rabbits experimentally infected with air-borne tuberculosis has provided a most convincing demonstration of the quantitative application of the apparatus to experimental pathology.

## DESCRIPTIVE

Approximate dimensions can be scaled from the drawing (Fig. 1). The infector nozzle is designed to give a flow of one sixth of a cubic foot of air under 20 pounds pressure. This evaporates approximately 1 cc of fluid in 10 minutes. Evaporation of only the very smallest droplets saturates this small air flow. The remainder are thrown out by the rapid whirling of the air and return to the pool of culture fluid. The resulting nuclei are finer than those with which we have had previous experience. The infector works well with quantities above 25 cc and will operate for several hours if the initial charge is as high as 50 cc.

The animal chamber (19 inches in diameter) seats in a seal of disinfecting solution. It is flexibly con-

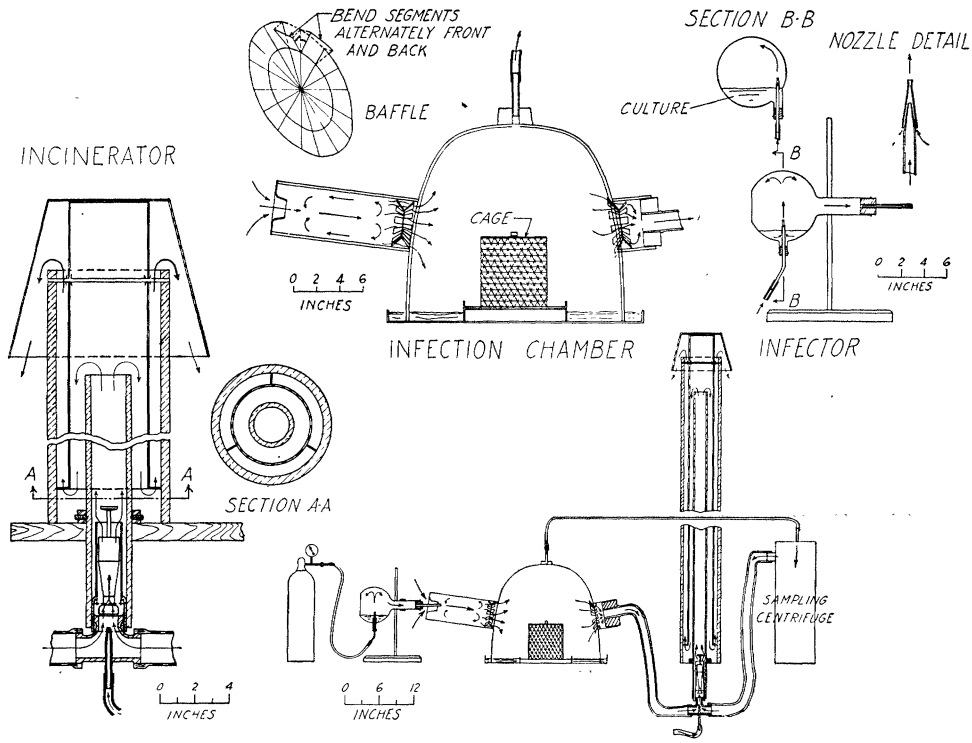


FIG. 1. An apparatus for the study of experimental air-borne disease. Center, exposure chamber; center left, infection flask; center right, incinerating chimney, which also provides flow through the device.

nected to the chimney, and can be lifted when the infector flask is removed from the orifice entrance. Simple baffles distribute the air evenly through the bell jar.

The incinerating chimney provides a flow of 3 cubic feet per minute of air through the system. The top of a Fisher burner, supplied by a special gas jet, supports vigorous combustion within the central 2-inch asbestos tube of the chimney. A central mushroom above the burner produces high turbulence in the combustion section of the chimney. The hot gases rise into an inverted galvanized tube, reversing the flow before entering the outer asbestos chimney. A simple air seal to prevent breakage of the draft is provided by inverting a pail over the top of the chimney. The air centrifuge is also exhausted into the incinerating chimney to avoid contamination of the room air. Ultra-violet lights irradiate the space around the apparatus so as to insure further the safety of operators. The greatest care must be exercised in design and operation to prevent any possibility of a back draft, but a year of experimental work has demonstrated the safety of the apparatus.

### Operation

A routine run would be conducted as follows: The burner is removed, lighted and reset in the chimney. Animal cages are placed on the platform by lifting the bell jar. The flask containing the culture is weighed and set in position with the nozzle venturi above the pool of liquid. Compressed air under five pounds pressure is admitted to the nozzle, and the flask turned until the culture flows into the stopper well and begins to atomize into the flask. The pressure on the nozzle is now raised to 20 pounds. The degree of air infection is determined by samples taken with the air centrifuge at appropriate intervals. To close a run, the nozzle is lifted out of the liquid by turning the flask, without cutting down the pressure. After 15 minutes, the air is cut off. Another 15 minutes is permitted to flush out the bell jar.

The precautions used in handling animals depends entirely upon the nature of the infection and the animals. They may be dipped in disinfectant; they may be jacketed. Sometimes no precautions are deemed necessary beyond handling the animals with gloves.

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## HALOWAX-PARAFFINE FOR INFILTRATING HISTOLOGIC TISSUES

Halowax No. 2020 is a hard, tough synthetic patented wax manufactured at the Wyandotte, Michigan, plant of the Halowax Division of the Bakelite Corporation. In reply to my request the manufacturer kindly furnished me the following information: Color, light yellow; flow point, 99–104° C.; viscosity, 34–38 at 150° C. (Saybolt); acid no., less than 0.1; penetration, 15–17; recommended using temperature, 130° C.; price 35¢ per pound F. O. B. Wyandotte, Michigan.

This wax is superior to bayberry wax for hardening paraffine, for the mixture supports the tissue as well as paraffine containing bayberry wax and the sections stick together better, thus forming a much stronger ribbon. My students and I find that difficult tissues, such as thyroid gland and adnexa, small intestine, rabbit appendix and kidney section readily at 4–6 micra after being infiltrated and imbedded in paraffine containing 12 to 15 per cent. Halowax. Refined household paraffine served as well as imbedding paraffine of 50–52° m. p. for this purpose. The tissues were infiltrated in tumblers under desk lights. No difficulties that could be attributed to the use of Halowax were encountered in ordinary microtechnique in which several stains and various methods of staining were used.

The chief technical disadvantages of Halowax-paraffine concerns the difficulty of getting the two waxes to mix without precipitation during cooling and the marked tendency of the mixture to lose its toughness after being used several times so that the tissue fails to ribbon. If the waxes are heated well above the flowing point of Halowax, removed from the burner and stirred frequently until the temperature has fallen to near the melting point, then poured into a tumbler and placed in cold tap water, the mixture usually cools without precipitation. Since xylene appears to be detrimental to this mixture, tissues are subjected to two or more changes of good paraffine before being infiltrated and imbedded in Halowax-paraffine.

Ed. D. Crabb

University of Colorado

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